OIPE JC105
MAY 27 2004
PATENT & TRADEMARK OFFICE

Docket No.: 28335/36996US
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Jeffrey S. Bartlett

Application No.: 10/038,972 Art Unit: 1636

Filed: January 4, 2002 Examiner: M. Marvich

For: AAV2 VECTORS AND METHODS

**DECLARATION OF JEFFREY S. BARTLETT, PH.D.**
**UNDER 37 C.F.R. § 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, the undersigned, hereby declare that

1. I am the inventor of the subject matter presently claimed in the above-identified patent application and make this declaration to provide information that I understand is relevant to an enablement rejection applied against the claims.

2. Currently, there are seven known AAV serotypes (AAV1, AAV2, AAV3, AAV4, AAV5, AAV7 and AAV8) while there were five known AAV serotypes (AAV1-AAV5) at the filing date of the application. The genetic structure and primary amino acid structure of the various AAV serotypes was known to be similar (Rutledge *et al.*, *J. Virol.*, 72: 309-319, 1998). For example, the overall amino acid sequence identity of AAV2 and AAV3 is 82% and the overall amino acid sequence identity of AAV2 and AAV4 is 75%. Further, the amino acid sequences of the capsid proteins are similar among the AAV serotypes. Despite their similarity, as disclosed in the application, these capsid proteins have several variable domains which were of interest for the insertion experiments, as a disruption within the variable regions was unlikely to effect capsid formation. The areas of variation are localized in corresponding regions of the capsid proteins within the different AAV serotypes.

Insertions within the corresponding variable regions in the capsid proteins of any AAV serotype are unlikely to affect the infectablity of AAV viral particles.

3. Even though the percentage of amino acid identity within the caspid proteins of the AAV serotypes is not striking, the similarity is biologically very significant because it results in similar three-dimensional structure among the capsid protein. As stated in the specification at page 11, three-dimensional structural analysis of five parvoviruses (canine parvovirus, feline parvovirus, minute virus of mice, parvo B19, and Aleutian mink disease parvovirus) was available at the time of filing. Thus, the means to carry out the three-dimensional structural analysis of AAV vectors was known at the time of filing. Similar three-dimensional structure is important for choosing insertion sites within the exposed loop regions (variable regions) of the capsid protein. Insertions within the exposed loops ensure that the peptide or target inserted in the vector is exposed to the surface of the virion to act as the appropriate target. Thus, the relative location of the loops and the site of epitope insertion in relation to the invariant regions that comprise the core is more important than the percentage of amino acid similarity.

4. The method by which insertion sites within the AAV2 capsid were chosen is described in Example 1 of the specification (pages 11- 18). The insertion sites were chosen based on the predicted secondary structure of the AAV capsid protein and were in regions expected to be exposed to the surface of the virion. The computer-based prediction of the secondary structure was based on the primary amino acid structure of the AAV2 capsid compared to solved virion structures of the five known parvoviruses. As displayed in Figure 1, the three-dimensional structure of AAV2 VP3 capsid protein is similar to the three-dimensional structure of the VP3 capsid proteins of canine parvovirus (CPV), feline parvovirus (FPV) and minute virus of mice (MVM).

5. Figure 2A shows the secondary structure of the AAV2 VP3 capsid protein as predicted from its primary amino acid sequence. To predict the three-dimensional structure, the following two computer algorithms were used: Chou-Fasman (*Adv. Enzymol Relat Areas Mol. Biol.* 47: 45-148, 1978), and Garnier-Robson (Levin *et al.*, *FEBS Lett.* 205(2):303-308, 1986; Biou *et al.*, *Protein Eng.* 2(3):185-91, 1988). These algorithms are available as components of several widely used DNA/protein analysis computer commercial

and freeware software packages. The analysis was carried out with the "Protean" component of the "DNAStar" software package. The structural determination was fit to the known structures of the related autonomous parvoviruses (MVM, FPV, etc.) as described in paragraph 4 above. The grey arrows identify regions of beta sheets, and squiggly lines represent alpha helices. Where the gray arrows are dashed, there was more uncertainty in the structural prediction. The yellow shaded area is a large area that didn't model well, aligned poorly with the known parvovirus sequences/structures, and was assumed to comprise a large surface-exposed region of the virus.

6. In light of the evidence available at the time of filing (Moskalenko *et al.* 2000. *J. Virol.* 74(4):1761-1766, 2000), the regions predicted to be exposed on the surface of the AAV2 VP3 capsid protein are depicted in green in Fig. 2B. The regions predicted to be exposed on the surface are in regions defined as flexible regions (loops between beta-sheets) outside the core beta-barrel domain (gray arrows). The actual secondary structure based on the currently available crystal structure, described in Xie *et al.*, PNAS 99(16) 10405-10410, 2002, is depicted in Figure 2C. The confirmed structure (Fig. 2C) demonstrates that the structure predicted at the time of filing was fairly accurate. Importantly, the predicted loops between the beta-sheets, outside the core domains (shaded areas) are very close to those present in the confirmed structure.

7. Figures 3 and 4 show computer modeled secondary structure of AAV1, AAV3, AAV4, and AAV5 VP3 caspid proteins compared to the known structure of the AAV2 VP3 capsid proteins taught in Xie *et al.* These models demonstrate that while different serotypes have different capsid primary amino acid sequences, the capsid proteins are each likely to assume nearly identical secondary structures. The serotypes have a common core (subunit fold) structure that is also shared by all parvovirus. The differences in the amino acid sequences are mainly present in the large surface-exposed loops, between the conserved regions (shaded in yellow in Figs. 3 and 4). The insertion sites of the present invention are within the regions of variation of the capsid protein. The residue numbering in Figs. 3 and 4 is a slightly off, accounting for differences in the size of surface exposed loops, but the core structure and most of the surface structure remains identical.

8. Figure 5 shows the location of several of the insertion sites taught in the specification in Table 1 (pp. 13-14) on the model of AAV VP3 capsid protein secondary structure. This model demonstrates that these sites map to the regions outside the core beta barrel domain, and within regions appearing in all variable AAV serotypes.

9. Thus, the specification provides information regarding the sites amenable to epitope insertion the capsid proteins of AAV2. This knowledge allows one to predict the regions within AAV1, AAV3, AAV4, AAV5, AAV7 and AAV8 that could support similar epitope insertions as discussed above. Methods such as computer modeling are routinely used by those of skill in the art to compare regions of epitope insertion in different serotypes. A simple alignment of the AAV capsid amino acid sequences allows one of skill in the art to transpose the site of insertion in AAV2 specifically taught in the application with corresponding sites in the other serotypes. The specification demonstrates that the region of amino acids 584-588 of the AAV2 VP3 capsid may be altered without loss of viral titer or infectivity. Figure 6 demonstrates that a simple alignment will reveal the corresponding regions of the various serotypes that will not be affected by insertions. This alignment method was used to select the sites for insertion for the experiments described in paragraphs 10 and 11.

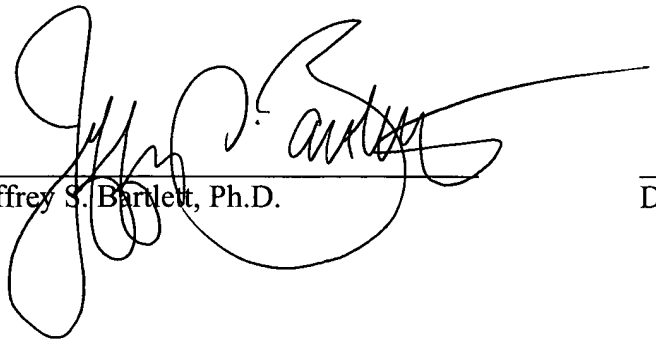
10. I supervised epitope insertion experiments carried out on various AAV serotypes where biotin acceptor peptides were inserted at insertion sites corresponding to those described in the application for AAV2. As described in the specification at pages 15-18 and in table 2, amino acids 584-588 of AAV2 can be altered without loss of viral titer or infectivity. The amino acid sequences of serotypes AAV1, AAV2, AAV3, AAV4, and AAV5 were aligned as described in paragraph 9 which allowed the for the identification of insertion sites that corresponded to the insertion sites described in the specification for AAV2. (See Fig. 6) Using the alignments, the biotin acceptor peptide (BAP), as described in the specification in Example 13 (pages 34-35) was inserted at the corresponding regions in the AAV vectors as shown in Fig. 6 by the open arrows.

11. The AAV-BAP vectors were generated by site-directed mutagenesis of plasmids containing the corresponding AAV serotype Rep and Cap open reading frames (ORF). Mutagenesis was confirmed by restriction endonuclease digestion. The altered Cap

genes were then substituted for the wild-type AAV serotype sequences in plasmid pACG2 to generate the mutant helper plasmids. Subsequently, the mutant AAV packaging plasmids were tested for their ability to generate AAV vectors with altered capsids by triple transfection with plasmid pAAV-LacZ (a plasmid containing LacZ flanked by AAV ITRs) and pXX6-80 (a plasmid containing Adenovirus helper DNA) according to established procedures. AAV vector preparations were assessed for particle formation. Particles were identified by ELISA using the A20 monoclonal antibody and DNA-containing particles were identified by dot-blot and/or PCR. As shown in Fig. 7 the insertion of BAP did not significantly decrease viral particle production in any of the AAV vector serotypes tested when mpared to the corresponding vector serotypes

12. The experiments described in paragraphs 10 and 11 demonstrate that epitope insertions at sites in various AAV vector serotypes corresponding to those described for AAV2 in the application are as effective insertions in those sites in AAV2. Thus, the disclosure in the specification enables one of skill in the art to carry out the epitope insertions claimed in AAV vector of any serotype.

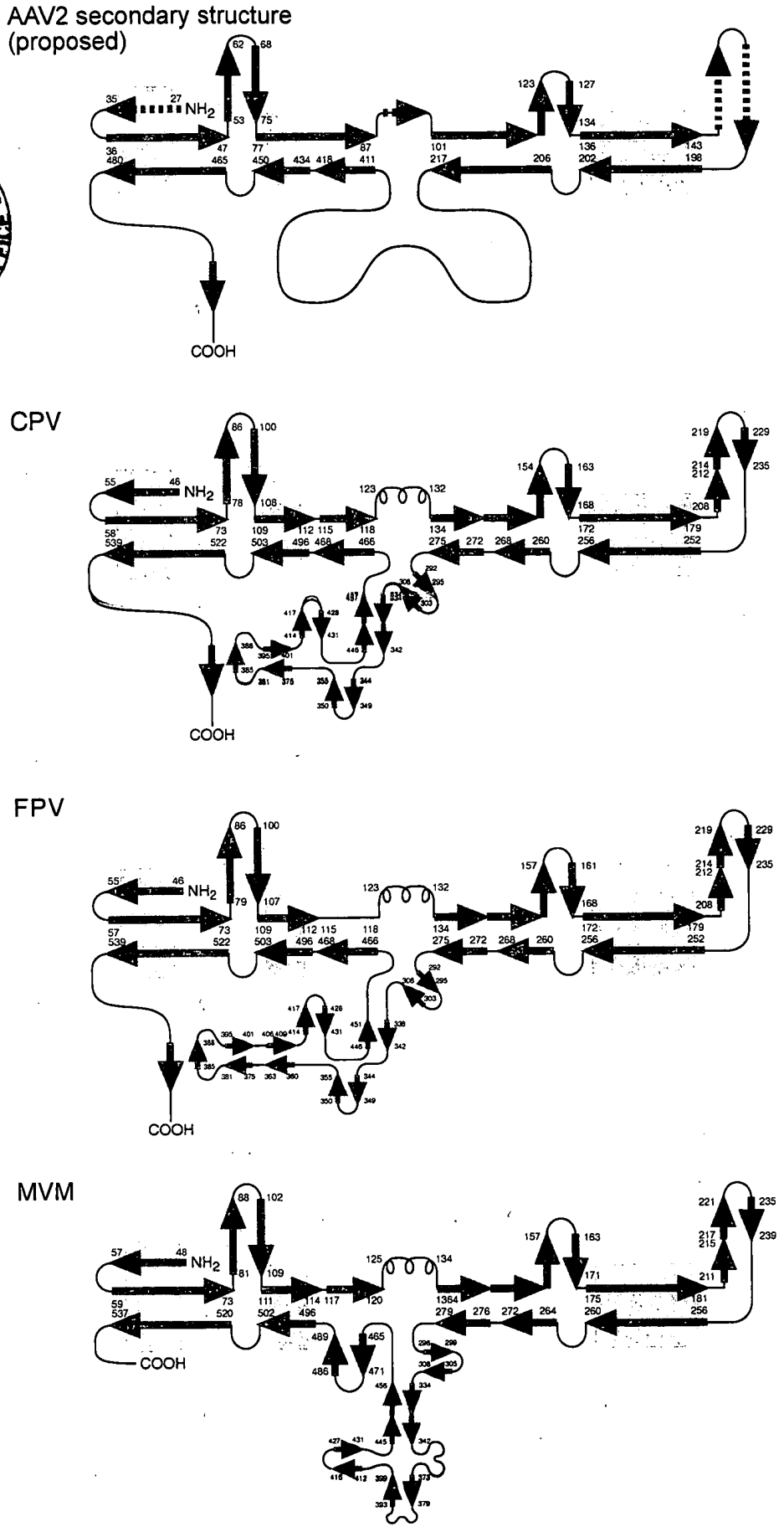
13. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.



Jeffrey S. Bartlett, Ph.D.

5/21/04
Date

# Figure 1




OIPE
MAY 27 2004
PATENT & TRADEMARK OFFICE
1600

OIPE
MAY 27 2004
PATENT & TRADEMARK OFFICE
JC105

Figure 2

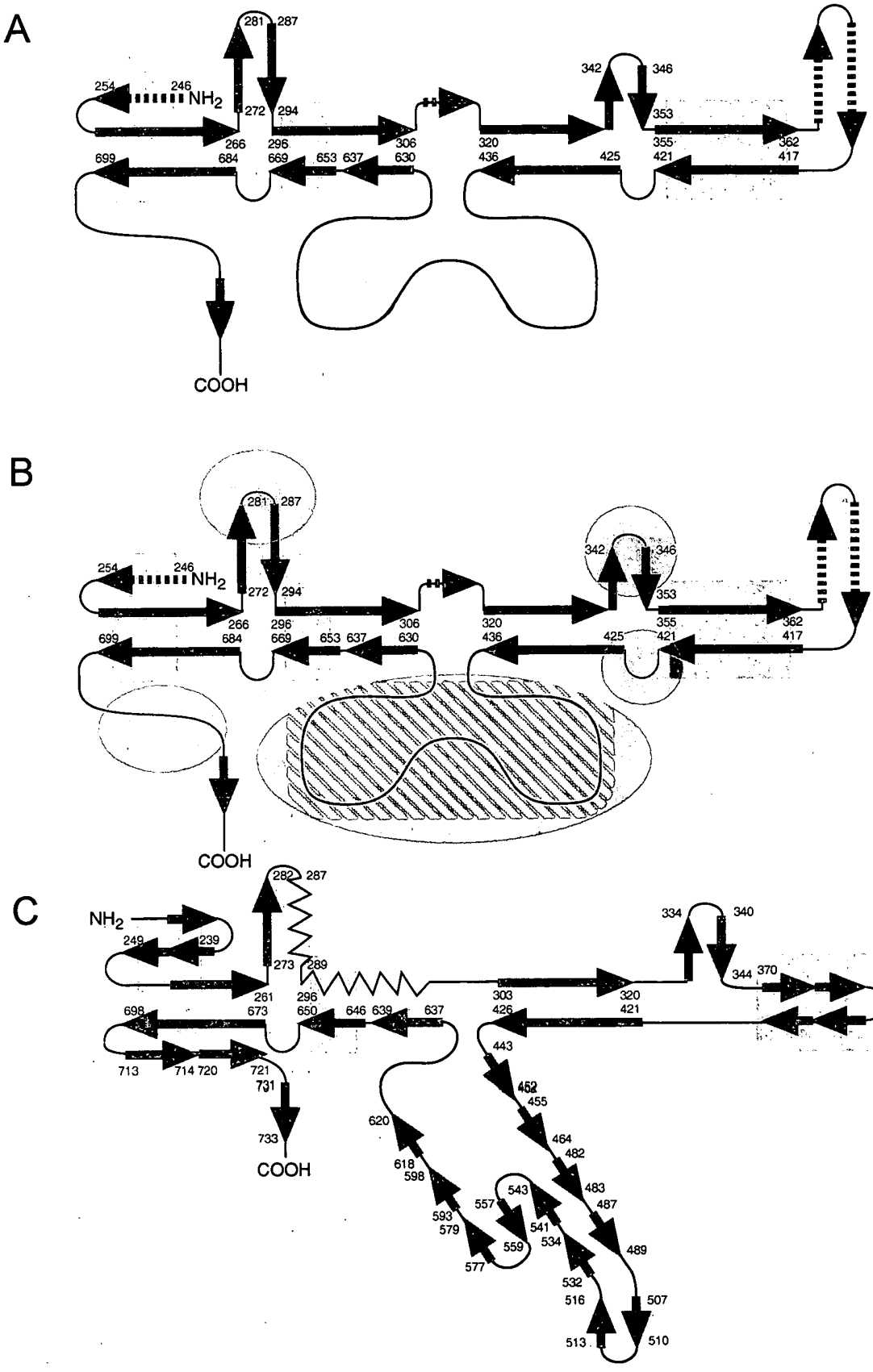
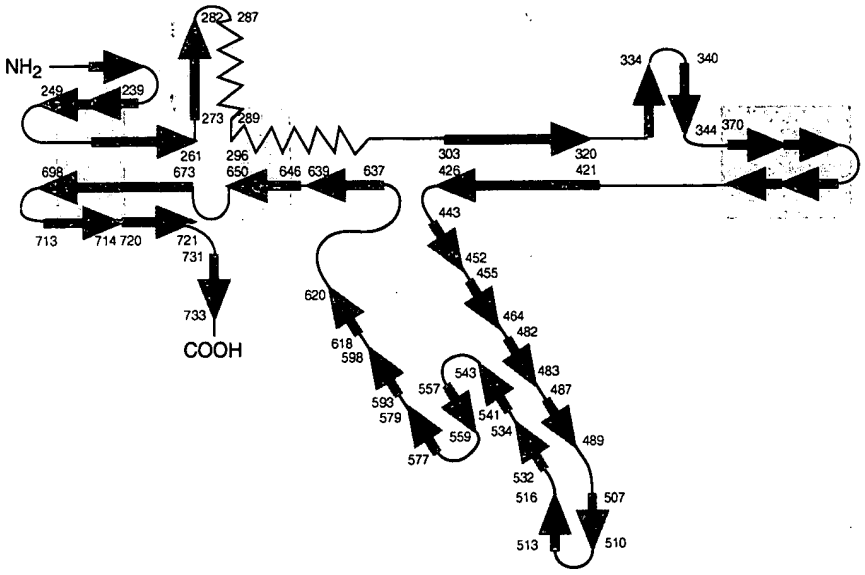


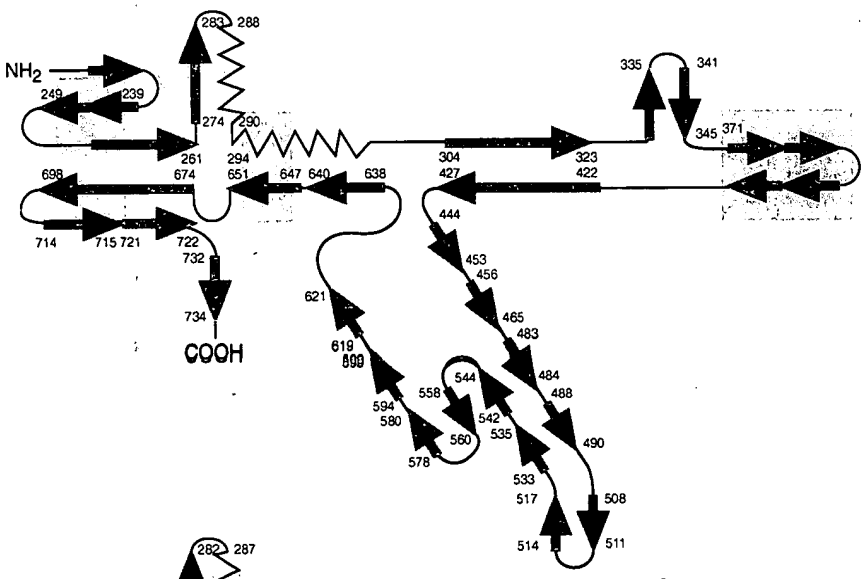
Figure 3

OIPE
MAY 27 2004
PATENT & TRADEMARK OFFICE
1600

AAV2




AAV1




AAV3

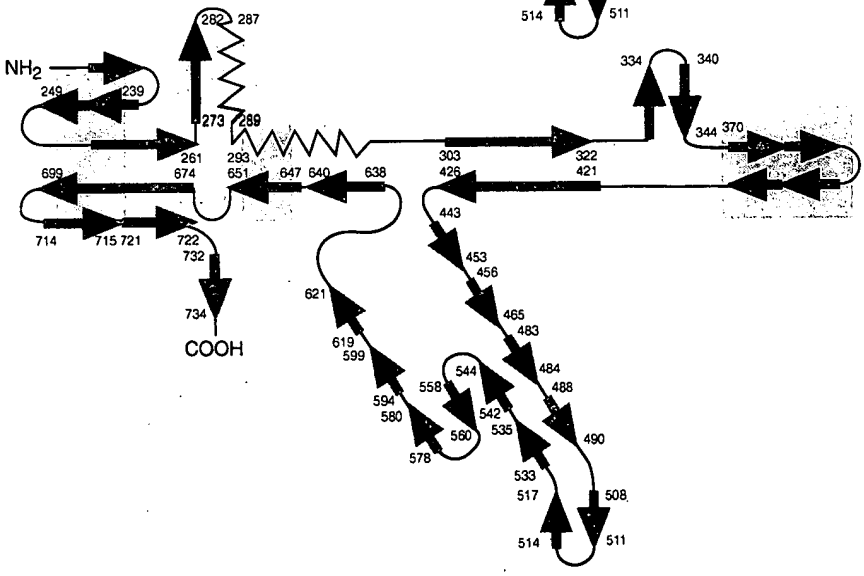
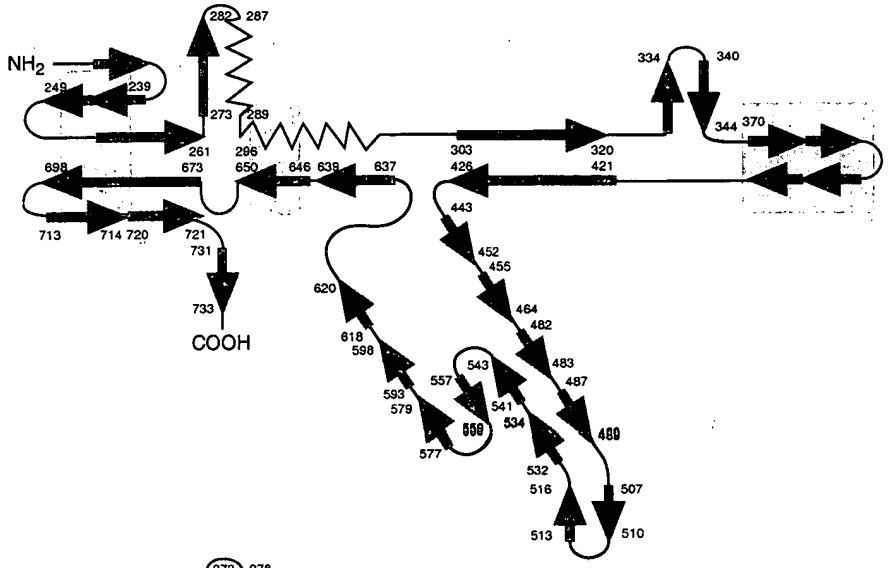


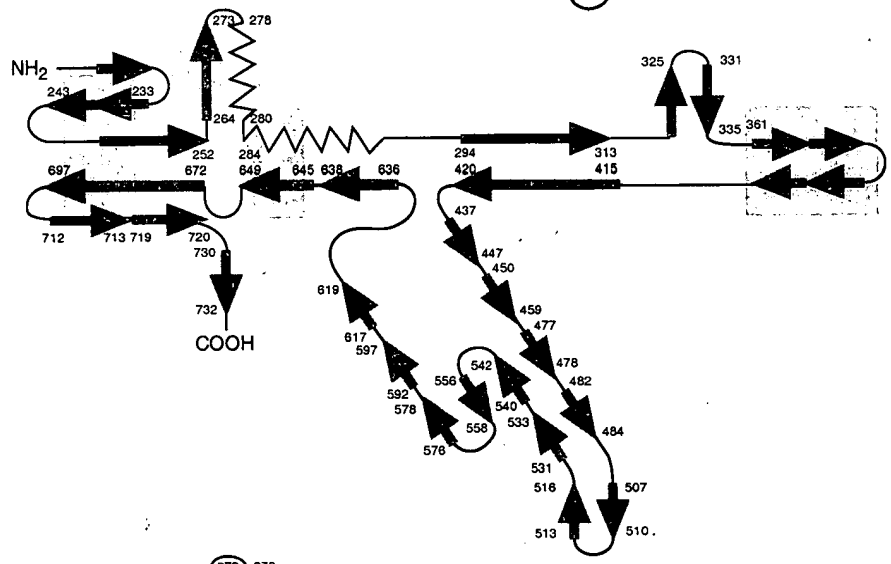
Figure 4

OIPE
MAY 2 7 2004
PATENT & TRADEMARK OFFICE

AAV2




AAV4




AAV5

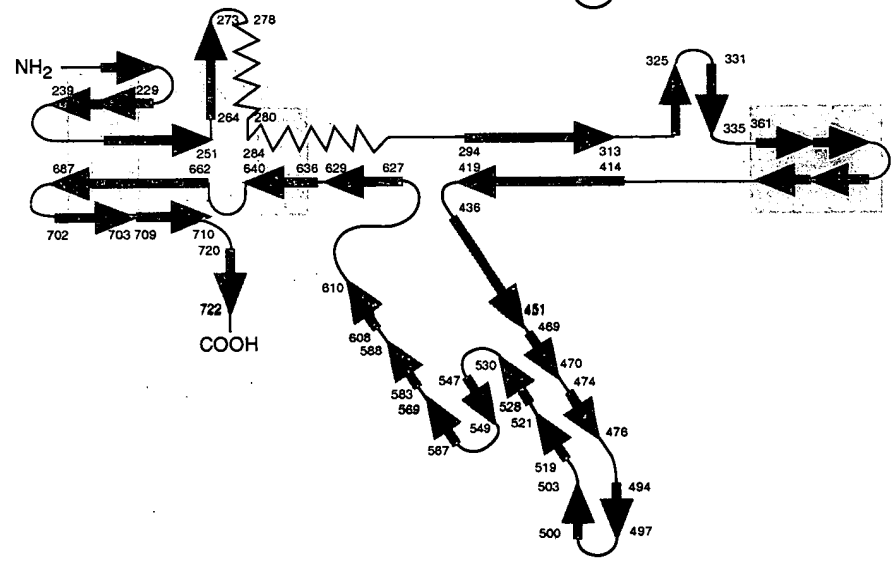
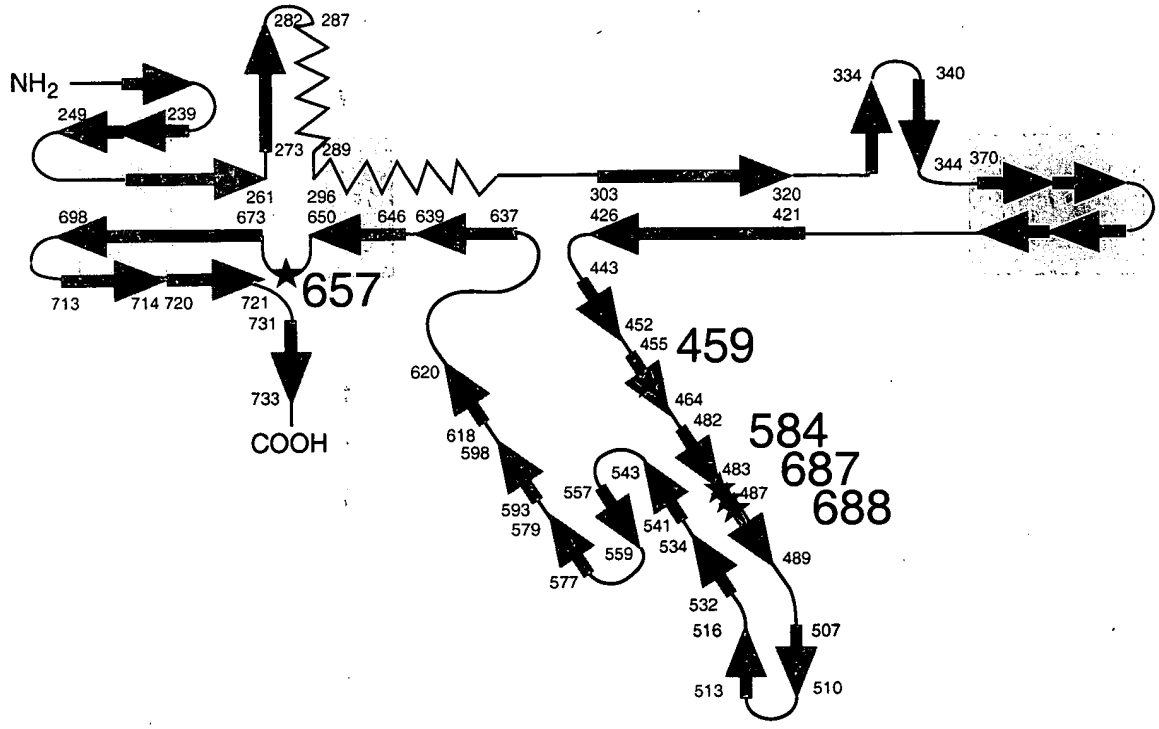


Figure 5

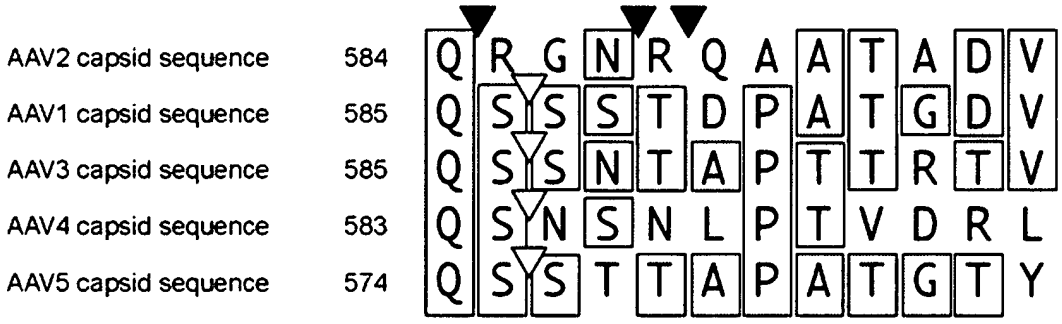
OIPE
MAY 27 2004
PATENT & TRADEMARK



# Figure 6

OIPE
MAY 27 2004
PATENT & TRADEMARK OFFICE

## AAV amino acid alignment




▼ Successful insertions within the AAV2 capsid protein (from previous work)

▽ Sites chosen for insertion within the other AAV capsid proteins

Figure 7

OIPE
MAY 27 2004
PATENT & TRADEMARK OFFICE
TC105